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ON THE SPECIFIC PRECIPITIN IN THE BLOOD OF PERSONS INJECTED WITH ANTIDIPHThERIC HORSE SERUM *

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Kraus,¹ in 1897, first demonstrated that the serum of an animal immunized against a foreign serum, when mixed with the immunizing serum, gives rise to a precipitate. A few years later, Hamburger and Moro,² v. Pirquet,³ and v. Dungern⁴ demonstrated in the serum of diphtheria patients, who had received antitoxic horse serum, the presence of a precipitin for horse serum, normal or antitoxic.

The method I have used in the study of this precipitin consisted in the macroscopical titration of serum, twenty-four hours old, from diphtheria patients, who had received therapeutic doses of antitoxic serum, against normal or antitoxic horse serum. In most tests, dilutions were made of the antigen, or horse serum, while the human serum was used undiluted; when the horse serum was undiluted and the human serum diluted, no marked variation in the results was obtained.

In order to economize on serum, small tubes with a lumen of about 3 mm. were employed. These were made by sealing in a Bunsen flame one end of thick glass tubes, about 6 cm. long. The thickness of the glass served somewhat to magnify the precipitin ring at the point of contact of the two sera. The antigens, consisting in majority of cases of normal horse serum, was first introduced into the tubes in various dilutions and they were arranged in a row in special racks, the depth being about 1 cm. Then, by means of fine pipettes, the human serum, in about equal amount, was run carefully into the bottom of the tubes in such a way that a definite line of contact was produced. The tubes were incubated at 37 C. for two hours, at the end of which time the results were noted.

At first, some confusion was encountered in the readings, because frequently the human serum was turbid, rendering the detection of a

* Received for publication November 3, 1914.

1. Wien. klin. Wchnschr., 1897, 10, p. 736.

2. Ibid., 1903, 15, p. 445.

3. Die Serumkrankheit, 1905, Vienna.

4. Die Antikörper, 1903, Jena.

precipitin ring difficult. It was found that dilution of the human serum with equal parts of salt solution increased the accuracy of the readings in such cases. McGowan⁵ has suggested that turbidity may be due to a lipemic condition, an anemic or an emaciated state. One or all these conditions frequently existed in my cases; the lipemic condition, because of the large amounts of milk and cream given the patients; the other states were results of the disease itself.

ANALYSIS OF PRECIPITIN CURVES

Welch and Chapman⁶ have advanced the idea that the precipitate is formed by constituents of the antigen and that there is a close rela-

TABLE 1
ANALYSES OF TWENTY-SIX CASES AS TO THE QUANTITY OF PRECIPITIN IN RELATION TO THE AMOUNT AND LOT OF ANTITOXIN SERUM USED

Case	Age of Patient	Lot of Antitoxic Serum	Units of Antitoxin	Cubic Centimeters of Serum Injected	Titer of Precipitin as Measured by Dilution of Antigen	Number of Days Between Last Injection of Serum and Appearance of Precipitin
634	25	S6	10,000	9	0	9
638	25	S8	10,000	7	0	6
641	6	S6	10,000	9	5	11
647	9	S8	30,000	21	10	9
628	23	440	40,000	27	20	5
630	40	S6	15,000	13.5	20	6
646	21	S8	10,000	7	20	11
637	6	S6	40,000	36	40	6
670	8	S6	20,000	18	40	9
624	5	S8	10,000	7	80	4
649	14	S8	5,000	3.5	80	8
651	10	S8	40,000	28	160	7
611	28	440	10,000	6.5	1,280	6
588	13	S6	35,000	31	1,280	7
570	37	S6	20,000	18	1,280	5
618	25	440	15,000	10	1,280	7
653	45	S8	10,000	7	2,560	5
597	7	S6	30,000	27	2,560	4
572	24	S6	15,000	13.5	2,560	7
599	16	440	35,000	24	5,120	7
536	18	S6	20,000	18	10,240	7
547	19	S6	25,000	22.5	20,480	7
591	22	S6	15,000	13.5	20,480	5
617	18	440	60,000	42	20,480	5
535	22	S6	40,000	36	20,480	8
578	8	S6	35,000	31	40,960	8

tionship between the quantity of the precipitate and the amount of antigen injected. Table 1, giving an analysis of twenty-six cases, demonstrates conclusively that the amount of precipitin present, as determined by the titer of the antigen, in which when mixed with human serum the precipitate appeared, is not dependable upon the

5. Jour. Path. and Bacteriol., 1909-10, 14, p. 395.

6. Brit. Med. Jour., 1910, 2, p. 1510.

amount of antitoxic serum injected, nor does there seem to be any relation between the amount of precipitin and the various lots of antitoxic serum used. In all cases the antitoxic serum was so-called concentrated or precipitated serum.

A study of the respective ages, sexes, and weights of the patients shows that these factors have little, if any, bearing on the amount of precipitin present. Thus, we are led to the conclusion that the precipitin content of the patient's serum is dependable upon intrinsic elements or processes of the patient's organism, the exact nature of which we, as yet, do not understand; and that it depends on the antitoxic serum only in that this substance, which consists of foreign protein, initiates or stimulates the formation, by the body cells, of that substance, which, when brought in contact with the antidiphtheric serum or normal horse serum, produces a precipitate. It is justifiable to infer that the blood serum of those patients, who showed the presence of precipitin in very high dilutions of antigen, as in Cases 535, 578, and 617 (Charts 1 and 2) who received 36, 31, and 42 c.c. of serum, respectively, would have shown a marked precipitin production following injections of much smaller quantities of antitoxic serum. From a study of serum disease in diphtheria, there has been noted a marked relationship between certain lots of antitoxic serum and the occurrence of the serum disease, but in respect to any relationship between the lot of antitoxic serum and the amount of precipitin produced in the patient's serum no relationship has been noted (Table 1).

Closely related to the foregoing point is the relationship between the duration of precipitin in the patient's serum and the amount and lot of antitoxic serum administered. Table 2 presents an analysis of twenty-three cases with special reference to this point. From a consideration of this table, it appears that the amount or lot of antitoxic serum has little, if any, influence upon the duration of precipitin for horse serum in the blood of the patients. Cases 647 and 646 demonstrate that, in certain individuals, a small dose of the same lot of antitoxic serum may give a longer appearance of precipitin than a larger dose. Case 647 received 30,000 units, or 21 c.c., of Lot S8 and the precipitin appeared for fourteen days; Case 646 received 10,000 units, or 7 c.c., of the same lot, and the precipitin appeared for seventeen days; Case 649, which received only 5,000 units, or 3.5 c.c., of the same lot, showed precipitin as late as the thirty-sixth day, when the patient was discharged; Case 628 received 40,000 units, or 27 c.c., of Lot 440 and showed precipitin only eight days, while Case 618, receiving 15,000

units, or 10 c.c., of the same lot, showed a high content of precipitin on the fifty-fourth day, when discharged (Chart 1).

An explanation, therefore, of the cause of the duration of precipitin in the blood rests, as does the explanation of the quantity of precipitin, upon certain intrinsic properties of the organism, the exact nature of which we do not as yet understand. At this point it is of interest to note that Hektoen⁷ has shown that the amount of antitoxic serum injected does not bear a definite relation to the amount of specific agglutinin, lysin, and opsonin produced.

TABLE 2

ANALYSES OF TWENTY-THREE CASES AS TO THE RELATION OF LOT AND AMOUNT OF ANTITOXIC SERUM TO INCUBATION PERIOD AND DURATION OF PRECIPITIN IN BLOOD OF PATIENTS

Case	Age of Patient	Lot of Antitoxic Serum	Units and Quantity of Antitoxin Injected		Number of Days Between Last Injection of Serum and Appearance of Precipitin	Duration in Days of Precipitin in Blood
628	23	440	40,000	27 c.c.	9	8
630	40	S6	15,000	13.5 c.c.	6	9+
634	25	S6	10,000	9 c.c.	9	11
647	9	S8	30,000	21 c.c.	9	14
597	7	S6	30,000	27 c.c.	4	17
646	21	S8	10,000	7 c.c.	11	17
637	6	S6	40,000	36 c.c.	6	18+
611	28	440	10,000	6.5 c.c.	6	19+
588	13	S6	35,000	31 c.c.	7	21+
641	6	S6	10,000	9 c.c.	11	21+
570	37	S6	20,000	18 c.c.	5	22
547	19	S6	25,000	22.5 c.c.	9	23+
572	24	S6	15,000	13.5 c.c.	7	26
578	8	S6	35,000	31 c.c.	8	28+
624	5	S8	10,000	7 c.c.	4	30
599	16	440	35,000	24 c.c.	7	31
591	22	S6	15,000	13.5 c.c.	5	33+
651	10	S8	40,000	28 c.c.	7	35+
649	14	S8	5,000	3.5 c.c.	8	36+
535	22	S6	40,000	36 c.c.	8	43+
617	18	440	60,000	42 c.c.	5	46+
536	18	S6	20,000	18 c.c.	7	53
618	25	440	15,000	10 c.c.	?	54+

Plus sign following the number of days signifies that precipitin was present at the time of discharge from hospital.

Average number of days per case: 26.

Average number of days for each lot: Lot S6, 23.9; Lot S8, 25; Lot 440, 31.6.

Von Pirquet³ suggests that the variability of the incubation, or latent period, may possibly be due to the amount or method of injection of the antigen. The results of this study, however, fail to show any connection of importance between the amount of antigen injected and the length of the incubation period (Table 2).

7. Trans. Chicago Path. Soc., 1907-9, 7, p. 223.

Case 630, receiving 15,000 units, or 13.5 c.c., of Lot S6, had an incubation period of six days; Case 637, receiving 40,000 units, or 36 c.c., of the same lot, also had an incubation period of six days; Case 570, receiving 20,000 units, or 18 c.c., of the same lot, had an incubation period of five days; Case 535, receiving 40,000 units, or 36 c.c., of the same lot, had an incubation period of eight days. A study of cases receiving Lot S8 shows a similar relationship.

The manner of administration of the antigen is of little value in this series, as in practically every instance the antitoxin was administered intramuscularly.

Charts 1 and 2 show more or less typical precipitin curves from the cases included in this study. The curves in Chart 2 show a feeble

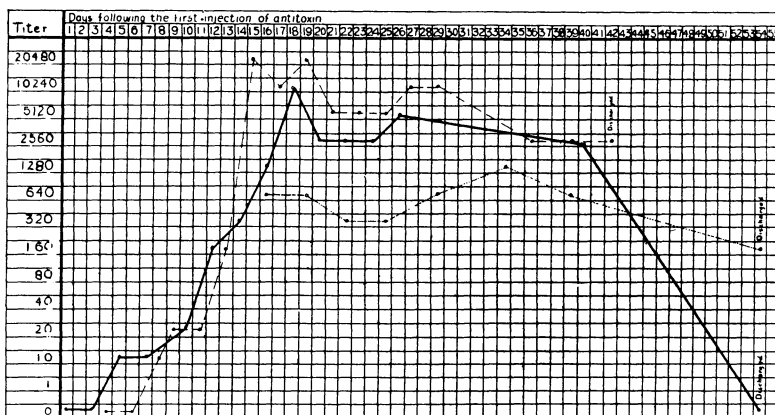


Chart 1.—Typical precipitin curves showing different phases. Small circles show the days on which the blood was withdrawn for titration.

Case 536 (heavy, solid line).—Male, age 18 years. Ill four days before receiving antitoxin, which was administered on three successive days, totaling 20,000 units, or 18 c.c., of Lot S6. No serum reaction was noted.

Case 535 (broken line).—Male, age 22 years. Ill three days before receiving antitoxin, which was administered on three successive days, amounting altogether to 40,000 units, or 36 c.c., of Lot S6. No serum reaction was noted. Complicated by typhoid fever.

Case 618 (dotted line).—Female, age 25 years. Received antitoxin on the day following the onset of diphtheria in one dose of 15,000 units, or 10 c.c., of Lot 440. Serum was not examined until the sixteenth day.

precipitin reaction in respect to quantity, but a strong reaction in respect to duration. Those in Chart 1 show a more marked reaction in both respects.

Von Dungern⁴ differentiates four phases in a typical precipitin curve. These phases are fairly well differentiated in the curves presented in the charts: Phase 1, or latent period, in twenty-five cases averaged 6.7 days, varying from 3-12 days; Phase 2, or ascending

phase of precipitin content, in eighteen cases averaged 5.1 days, varying from 3-15 days; Phase 3, or antibody plateau, in seventeen cases averaged 16.3 days; Phase 4, or decline of antibodies, could not be followed to the end in all my cases because of the discharge of the patients from the hospital before this point was reached. In a few cases, however, where it was possible to follow the precipitin curve to a completion, this phase was found to be short, averaging from 3-6 days.

Von Dungern's differentiation was made with precipitin in rabbit serum, consequently a close comparison of the duration of each phase with precipitin in human serum is of little value. However, the time in each case varies but little. Von Dungern's time for each phase was as follows: Phase 1, 4.5-5.5 days; Phase 2, about 2 days; Phase 3, variable, 3-9 weeks; Phase 4, variable, usually a few days.

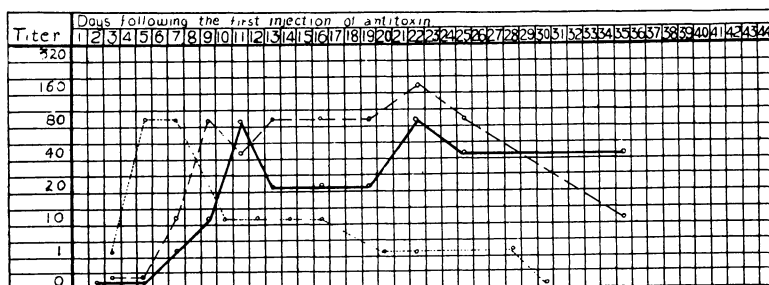


Chart 2.—Showing low precipitin content with typical curves. Small circles designate days on which blood was withdrawn for examination.

Case 649 (heavy, solid line).—Male, age 14 years. Antitoxin amounting to 6,000 units, or 3.5 c.c., of Lot S8 was given seven days after the onset of the disease. No serum reaction.

Case 651 (broken line).—Male, age 10 years. He was given 40,000 units of antitoxin, or 28 c.c., of Lot S8 four days after the onset of the disease. Urticaria appeared on the third day after the antitoxin was given and disappeared on the eighth day.

Case 624 (dotted line).—Male, age 5 years. Antitoxin amounting to 10,000 units, or 7 c.c., of Lot S8 was given three days after the onset of the disease. No serum reaction occurred.

It may be noted that, on the whole, the precipitin curves in my cases follow very closely the typical antibody curves, and especially do they follow the curves obtained by Hektoen⁷ for opsonin, agglutination and lysin for horse corpuscles in blood of man injected with antidiphtheric horse serum.

SERUM DISEASE

Hamburger and Moro² suggest a fundamental connection between the appearance of precipitin in the blood of diphtheria patients, who have received antitoxic serum, and the appearance of serum disease.

While they reject several theories which have been entertained concerning the etiology and genesis of the serum disease, no satisfactory explanation is advanced by them.

A number of cases in this study presented a definite precipitin curve and, at the same time, typical cutaneous serum reactions. From a study of the course of the serum reactions in these cases and its effect on the precipitin curve, there is some indication of a connection or a relationship between the serum disease and the precipitin content of the patient's blood.

Of the twenty-seven cases, nine manifested a recognizable serum disease. Analyses of some of these cases are interesting.

Case 617.—The precipitin curve shown in Chart 3 demonstrates a certain connection between the serum disease and the precipitin content of the serum. On the seventh day following the injection of anti-toxic serum, while precipitin was demonstrable in a dilution of 640 of the antigen, urticaria of a typical form developed with other recognized signs of serum disease which continued until the thirteenth day. In the midst of this intervening period there occurred on the tenth day a marked fall in the precipitin content to a dilution of 80 of the antigen, which fall was followed on the thirteenth day, the day on which the urticaria faded, by a sudden rise in the precipitin to a dilution of 20,480 of the antigen, which dropped on the fourteenth day to a dilution of 1,280, where it remained constant for a number of days.

Cases 599 and 578 (Chart 3) and Case 651 (Chart 2).—These cases demonstrate the same general connection, except that in the curves of these cases there is not present a sudden or marked drop in precipitin content with the onset or during the course of the serum disease, but there is shown a marked rise in each precipitin curve synchronous with the disappearance of the serum disease.

Case 646.—The precipitin curve of this case is not recorded on the charts, as the patient developed urticaria which faded before precipitin was demonstrable in the patient's serum.

From the observations in these five cases, we are warranted in drawing the conclusion that the serum reaction is connected with a union of the precipitin element with some other element in some way not apparent or understood, and that the disappearance of the urticaria and other symptoms of serum disease is synchronous with the liberation of the precipitin from this union, resulting in a sudden rise in the precipitin content of the blood. The extent of the fall in the pre-

cipitin content at the onset of the serum disease would seem to be dependent on the rapidity of precipitin formation compared to the rapidity of its union or consumption for the production of the phenomenon of the serum disease.

By some it has been intimated that the visible manifestations of serum reaction, and particularly the cutaneous manifestations, are the result of an actual precipitation in the blood, which results in the plugging of small capillaries; such precipitation would also result in the removal of a certain amount of demonstrable precipitin from the free,

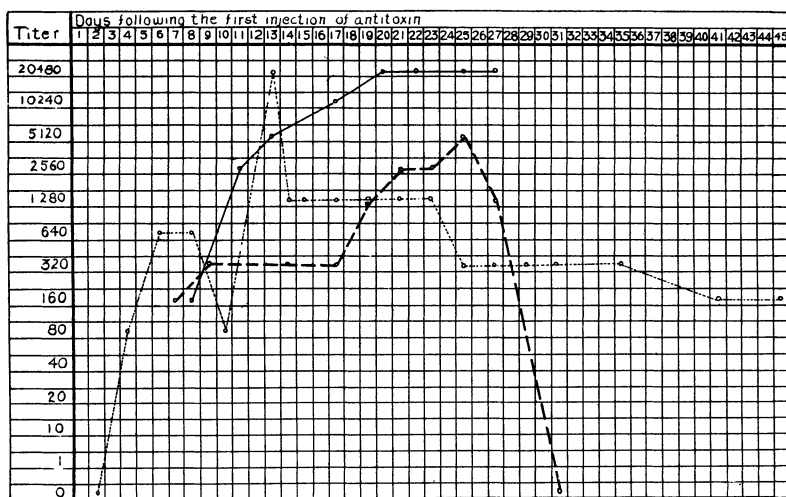


Chart 3.—The effect of serum reaction on precipitin content in the blood serum, also atypical precipitin plateaus.

Case 578 (heavy, solid line).—Male, age 8 years. First received antitoxic serum on day following onset of disease, altogether 35,000 units, or 3 c.c., of Lot S6 were given. Serum reaction, as manifested by urticaria, appeared on the third day, and disappeared on the seventh day.

Case 599 (broken line).—Female, age 16 years. Antitoxic serum first administered on the day of the onset of the disease and additional doses were given on three succeeding days, until a total of 35,000 units, or 24 c.c., of Lot 440 was given. Serum reaction, consisting of urticaria, appeared on the tenth day and disappeared on the fourteenth day.

Case 617 (dotted line).—Female, age 18 years. Antitoxic serum first given two days following onset of disease. Additional doses given on two successive days, until a total of 61,000 units, or 42 c.c., of Lot 440 was given. Serum reaction appeared on the seventh day and disappeared on the thirteenth day.

circulating blood. Whether or not this is the explanation of the apparent relationship between the precipitin content and the serum reaction has not been settled in this study, but late observations on this point by Hamburger and Moro² have seemed to disprove the possibility of a plugging of capillaries with a demonstrable precipitation in the blood.

While no special study of the phenomenon was undertaken in this series of cases, it is interesting to note that nine of twenty-seven cases showed a serum reaction; and that in these nine cases the incubation, or latent period averaged 9.75 days, which agrees closely with the results obtained by Weaver,⁸ who found an incubation period of 8.4 days in 801 cases of serum disease, and Cowie,⁹ who found an average of 5-7 days.

SUMMARY

The amount of the precipitin, measured by the degree of dilution of the antigen in which the precipitin is demonstrable, is not dependent on the amount or lot of antitoxic serum administered, nor on the age, sex, or weight, of the patient. Neither is the duration in days of precipitin in the patient's serum dependent on any of these factors.

The amount and possibly the duration of precipitin in the blood are dependent on some, as yet unknown, intrinsic process of the organism, which, however, is initiated or activated by the introduction of foreign protein in the antitoxic serum.

There is no evidence in this series to indicate that the length of the incubation, or latent period of the precipitin element is influenced materially by the quantity or method of administration of the antitoxic serum.

The various phases of the precipitin curve in this series compare favorably with the length of the respective phases of the antibody curves.

Precipitin is utilized or withdrawn from the blood during the course of the serum disease, and possibly is a factor in the production of the various phenomena of serum disease.

8. Arch. Int. Med., 1909, 3, p. 485.

9. Am. Jour. Dis. Child., 1914, 7, p. 253.